

Experiment No.8

pH DETERMINATION OF BODY FLUIDS BY DIFFERENT METHODS

pH indicators:

By using pH indicators an approximate value of pH of a solution can be obtained. The indicators dissociate like a weak acid on coming in contact with solution and give range of colours depending on pH. Dissociated and undissociated form of the indicator has different colours.

pH papers:

pH papers coated with indicators of different pH range are commercially available.

Procedure

Cut small pieces of the pH paper and place them on glass slide. Dip the glass rod in different body fluids of unknown pH one by one and touch the papers. Note the change in colour of pH papers for different body fluids. Match the colours with the colours of standards given the container of pH papers.

pH meter:

Accurate pH can be measured using pH meter which measures e.m.f. of a concentration cell developed from a reference electrode, test solution and a glass electrode sensitive to H^+ ions. The combined electrode constituting of glass and reference electrode is dipped into the test solution for accurate measurement of pH.

Procedure

The instrument is calibrated with the known buffers supplied by the supplier or with the primary buffers prepared for this purpose. Usually the buffers prepared are of pH 4, 7 and 10. After the preparation with the known buffers pH of unknown is determined.

Buffers can be made in stock solutions and these are diluted before use. Preparation of some of buffers frequently used in Biochemical studies is given below. Unless otherwise stated by following these procedures 0.1 M buffer will be obtained.

a) Acetate Buffer

Stock solution

- A) 0.2 M acetic acid (11.55 ml/L)
 B) 0.2 M sodium acetate (16.4 g of sodium acetate or 27.2 g of sodium acetate 3H₂O per L)

x ml of A + y ml of B, diluted to a total volume of 100 ml

	X	Y	pH
1	46.3	3.7	3.6
2	41.0	9.0	4.0
3	30.5	19.5	4.4
4	25.5	24.5	4.6
5	10.5	39.5	5.2
6	4.8	45.2	5.6

b) Phosphate Buffer

Stock solution

- A) 0.2 M monobasic sodium phosphate (27.8 g in 1 L)
 B) 0.2 M dibasic sodium phosphate (53.65 g of Na₂HPO₄ · 7H₂O or 71.7 g Na₂HPO₄ · 12 H₂O in 1 L)

x ml of A + y ml of B diluted to a total volume of 200 ml

X	Y	pH
93.5	6.5	5.7
85.0	15.0	6.1
68.5	31.5	6.5
56.5	43.5	6.7
16.0	84.0	7.5
5.3	94.7	8.0